IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of
SVANBORG ET AL

Atty. Ref:. **032313-003**

Serial No. **09/555,270**

Group: **1642**

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Examiner: **Holleran**

For: **THERAPEUTIC AGENTS**

CONSIDERED
RH
4/21/2003

DECLARATION

I, Catharina Svanborg, of St Månsgatan 4, 222 29 LUND, Sweden, hereby declare as follows:

1. I was awarded the degree of MD from the Medical Faculty of Göteborg University in 1974, followed by the degree of PhD in 1978. In 1979, I became Associate Professor, in the Department of Medical Microbiology, also at Göteborg University, and thereafter became a Specialist in Clinical Immunology in 1984, and a Specialist in Clinical Bacteriology in 1988. Since 1989, I have been Professor of Clinical Immunology at Lund University. During my career, I have received a number of Honours and Awards including the Domagk Award, presented by Bayer International,in 1992, the Windemere Award of the British Pediatric Society in 1996, and the Annual Jubilee Awards of the Swedish Medical Society in 1997. I have been a Fellow of the Royal Swedish Academy of Sciences since 1996, and have been named as author on some 400 publications on various areas of my work. In addition, I am an inventor of the subject patent application.

2. As inventor of the subject patent application, I am aware of the objections that are being raised against the application by the Examiner of the US Patent and Trade Mark Office. I understand that there is a question over whether the multimeric α-lactalbumin as described by Sabharwal et al. in WO 96/04920 and Hakansson et al. Proc. Natl. Acad. Sci. USA 92:8064-8068 (1995) may be a protein complex with a cytotoxic agent that had not been characterised.

3. I am a co-author on both of the references mentioned in section 2 above. In spite of extensive research on this product, we have never observed any unexplainable products or compounds present within the multimeric α-lactalbumin obtained as described in these references, which might represent a cytotoxin. Furthermore, as explained on page 10 of WO96/04920, we were able to produce multimeric α-lactalbumin with a similar or better biological activity from commercial human α-lactalbumin, by subjecting this to similar ion exchange chromatography, using a high (1M NaCl) salt gradient. In my opinion, this demonstrates that the biological activity is not a function of an uncharacterised cytotoxin present in milk.

4. Following on from the work described in these references, we conducted more detailed structural analyses of the proteins described there, and came to the conclusion that the biologically active protein described in the Sabharwal et al. Hakansson et al. references listed in section (2) above is a folding variant of α-lactalbumin, which is stabilised by a co-factor such as oleic acid, which is present in casein.

In order to prove this hypothesis, we produced recombinant α-lactalbumin, by expression in *E. coli*. This protein was therefore free of any other components found in the casein-containing fraction of milk. We were then able to convert this pure protein to the biologically active form by first partially unfolding it using EDTA to remove calcium ions and to form the apo protein, and passing this down a column matrix which had been pretreated with oleic acid. This further work has been reported in P.N.A.S. 2000, 97, 8, 4221-4226, a copy of which is enclosed as **Annex A**.

The product of this process had the same biological and physical properties of the product obtained by Sabharwal et al. and Hakansson et al. and is, in my view, the same product. As reported in the paper, oleic acid and other lipid extracts at the concentrations found in this product was tested and found to have no cellular effects. Therefore it is clear that the oleic acid is not acting as a cytotoxin. Neither is oleic acid a label reagent.

5. It is clear to me that the product obtained and reported in the references does not include an uncharacterised cytotoxin. The biological effects observed was the result of the existence of the new folding variant of α-lactalbumin, which was induced in the references as a result of the fractionation and purification treatments to which the milk was subjected. In particular the ion exchange chromatography, combined with elution at high salt concentration and the presence of the co-factor, resulted in the conversion of α-lactalbumin to the biologically active folding variant.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Svanborg

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Catharina Svanborg

Jan 03

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Date